GROWTH-INCREMENT FORMATION USING OTOLITHS AND SCALES OF JUVENILE CHINOOK SALMON

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A

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Abstract

Freshwater growth of juvenile Chinook salmon *Oncorhynchus tshawytscha* influences survival and recruitment to the adult population. Retrospective analysis is used to measure salmon growth at previous ages, with fish size and growth assumed to be accurately reflected by otolith increments and scale circuli. I conducted a 122-d laboratory experiment to validate the relationship among body size, growth, and width to daily otolith growth increments and scale circuli in juvenile stream-type Chinook salmon. Fish total length was found to be proportional to otolith axis length ($r^2 = 0.209$, $p < 0.001$), otolith diameter ($r^2 = 0.667$, $p < 0.001$), and scale radius ($r^2 = 0.538$, $p < 0.001$). Somatic growth was accurately reflected by growth in otolith axis length ($r^2 = 0.65$, $p < 0.001$) and growth in scale radius ($r^2 = 0.449$, $p < 0.001$). My study validated the assumption that fish body size and growth are reflected by otolith and scale size and growth-increment formation. The findings of my study can be used to ascertain body size at previous ages, which will help managers detect threshold sizes, examine the strength of size-selective mortality, and determine how growth rate affects smolt migration, early marine survival, and duration of ocean residency.

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Introduction

Chinook salmon *Oncorhyncus tshawytscha* occur throughout the Kuskokwim and Yukon river drainages in Alaska (ADF&G CRST 2013). The Kuskokwim River supports nearly half of the total annual subsistence harvest of Chinook salmon in Alaska, with Yukon River Chinook salmon providing important commercial and subsistence fisheries in both the United States and Canada (Howard et al. 2009; Molyneaux et al. 2010; ADF&G CSRT 2013). Adult returns of Chinook salmon to both rivers have declined over the past 20 years, with recent runs being some of the lowest on record and resulting in gear restrictions, shortened seasons, and fishery closures (Schindler et al. 2013; ADF&G CRST 2013). Because of the commercial and subsistence importance of Chinook salmon in interior Alaska (Linderman and Bergstrom 2006; Howard et al. 2009), fisheries managers and stakeholders have engaged in research to determine a cause or causes of the poor returns. Recent studies have used retrospective growth analysis to determine how freshwater growth affects survival and recruitment of juvenile Chinook salmon to the adult population in hopes of explaining the recent declines in abundance (Ruggerone et al. 2009; Leon 2013).

Retrospective analysis, or back-calculation, is the reconstruction of size at an earlier age for a species by determining the relationship between the size of a bony structure (e.g., the otolith or scale) at a given length over a broad range of fish sizes (Fraser 1916; Casselman 1987; Campana 1990; Fisher and Pearcy 1990; Francis 1990; Pierce et al. 1996; Wootton 1998; Klumb et al. 1999; Klumb et al. 2001). Once a model has been established, back-calculation of a fish's length at a previous age is estimated by aging a bony structure and measuring the radius of the structure to the specific growth increment or annulus in question (Holtby et al. 1990; Baumann et al. 2013). The length of the radius is entered into the model, and the result approximates the fish's length at a previous age (Baumann et al. 2013). Most fish species (including Chinook salmon)

deposit regular increments on hard body structures, including fin rays (Zymonas and McMahon 2009), otoliths (Secor and Dean 1992; Bestgen and Bundy 1998; Zabel et al. 2010), and scales (Fisher and Pearcy 1990; Fisher and Pearcy 2005; Ibanez et al. 2008). Increments on bony structures may be deposited daily in the case of otoliths (Bradford and Geen 1992; Bestgen and Bundy 1998; Sakaris and Irwin 2008) or increment-deposition frequency may be related to growth rate, as in the case of scales (Beamish et al. 2004; Ibanez et al. 2008). For both structures, the width between growth increments has been found to be correlated with somatic growth rate (Fisher and Pearcy 1990; Bradford and Geen 1992; Wootton 1998; Beamish et al. 2004). Annual variations in growth rate from relatively rapid growth (spring and summer) to comparatively slow growth (fall and winter) produce alternating patterns of widely and closely spaced increment groups on both otoliths and scales (Fukuwaka 1998; Wootton 1998; Moyle and Cech 2004). Fish age in years is estimated by counting closely spaced increment groups, known as annuli (Campana 2001; Moyle and Cech 2004).

The otolith of a fish is composed of inorganic material, most often calcium carbonate, in an organic matrix consisting of proteins (Pannella 1971; Campana 1999). Teleost fishes have three pairs of otoliths (the sagittae, lapilli, and asterisci), with the sagittal otoliths being the largest in most species (Campana and Neilson 1985). Otoliths are part of a fish's inner ear, and each otolith is housed separately in a semi-permeable sac containing endolymphatic fluid (Secor and Dean 1992). The otolith is immersed in the endolymph, from which new layers of calcium are periodically deposited onto the outer otolith surface (Campana and Thorrold 2001). The deposition of a new layer occurs daily in many fishes, forming a bipartite growth increment of light calcium crystals and dark protein (Campana and Neilson 1985; Bradford and Geen 1992; Moyle and Cech 2004).

Otoliths, especially sagittae, act as sound detectors (Popper and Coombs 1980). Fish approximate the density of water, and sound waves pass through fish as the waves move through the water column. Otoliths are dense structures, and sound waves cause the otolith to move at a differential amplitude and phase than the body of the fish (Popper

and Lu 2000). Sound waves striking the otolith cause the structure to move slightly, which bends small groups of cilia bundles underlying the otolith and stimulates the inner ear (Popper and Coombs 1980; Popper and Lu 2000; Popper et al. 2005). In much the same way, otoliths allow the fish to maintain balance because motion and orientation of the fish cause the otolith to move against cilia bundles, transmitting vestibulary information to the fish's inner ear (Popper et al. 2005). While the primary function of sagittae is detecting sound, lappilar otoliths are responsible for maintaining balance and orientation (Riley and Moorman 2000).

Otolith growth rate is influenced by a number of factors, including temperature and food availability (Bestgen and Bundy 1998), but has been found to be correlated with somatic growth (Bradford and Geen 1992; Secor and Dean 1992; Strelcheck et al. 2003; Sakaris and Irwin 2008). Otolith increment formation tracks the daily variation in somatic growth rate through the width between each increment. For example, during periods of rapid growth, increments are more widely spaced than during periods of slow growth (Wootton 1998; Moyle and Cech 2004). The relationship between otolith and somatic growth is conservative, and can be insensitive to variations in growth rate over short periods of time (Bradford and Geen 1992; Secor and Dean 1992). Otolith growth has also been shown to continue through periods of starvation, which may distort the relationship between the width between otolith increments and fish growth rate (referred to as uncoupling or decoupling; Maillet and Checkley 1990; Bradford and Geen 1992; Wright et al. 2001). Regardless, the width between increments, otolith radius, and otolith diameter have been found to be correlated with overall body size (length) of a fish (Secor and Dean 1992; Harvey et al. 2000; Strelcheck et al. 2003).

Similar to otoliths, the width between scale circuli has also been found to reflect somatic growth rate (Healey 1982; Wootton 1998; Beamish et al. 2004; Moyle and Cech 2004) and overall scale size (radius) has been correlated with fish length (Fisher and Pearcy 1990; Holtby et al. 1990). Scales form a partly overlapping barrier that protects the fish from minor injury (Frietsche and Bailey 1980; Metz et al. 2012), and each scale must grow in proportion to somatic growth to prevent gaps from opening along the scale

barrier. Fish scales grow through accretion of collagen by fibroblast cells located near the outer edge of the scale along with calcification of the outer surface (Moyle and Cech 2004). Circuli are concentric ridges on a scale's upper surface, forming in the intercellular space between two overlapping osteoblasts during scale growth as the osteoblasts at the margin of the scale are flattened and incorporated into the inner portion of the scale (Fukuwaka 1998).

Scale circuli appear to form as the result of pressure on the scale created as the scale grows in size and is restricted by the surrounding scales and tissues (Yamada 1961, 1964, 1971). This pressure causes a ridge (the circulus) to form near the scale margin, and restricts expansion of the scale until somatic growth alleviates the pressure and allows normal scale growth to proceed beyond the newly formed circulus (Yamada 1964). Scale circuli are not formed daily, but circuli formation appears to be closely linked with somatic growth rate (Beamish et al. 2004; Ibanez et al. 2008). Circulus formation can also be influenced by fish age, size, and reproductive status (Fisher and Pearcy 1990), as well as water temperature, food, and photoperiod (Bilton 1970; Bilton and Robins 1971a, 1971b). Because of the many influences on otolith and scale growth and increment formation for both structures, the relationship between size and growth of the fish and size and growth of its hard structures must be validated.

Validation is the repeated comparison of the relationship between otolith or scale increment formation and somatic growth in a species or stock over a period of time using fish of known age, and is critical for accurate use of back-calculation methods (Francis 1990; Wootton 1998). There are three criteria for validation: (1) the current radius of a scale increment must be the same as the radius of the increment at formation (Beamish and McFarlane 1983); (2) the assumed time of increment deposition must be accurate (e.g., the scale or otolith has been aged correctly; Campana 2001); and (3) the model selected must appropriately relate the increment radius to body size for the fish species being tested (Pierce et al. 1996; Klumb et al. 1999). Proper validation ensures that back-calculated lengths are representative of the study population, as many back-calculation

models exist and must be selected based on how they fit the species or stock of interest (Francis 1990; Pierce et al. 1996; Klumb et al. 1999).

Fish body size is assumed to be correlated with otolith and scale size, with somatic growth rate reflected by the width between otolith daily growth increments and scale circuli (Fisher and Pearcy 1990; Holtby et al. 1990; Bradford and Geen 1992; Fukuwaka and Kaeriyama 1997; Beamish and Mahnken 2001; Beamish et al. 2004). Few studies have attempted to validate the relationship between fish size and growth and the size and growth of otoliths and scales (Fisher and Pearcy 1990; Bradford and Geen 1992; Fukuwaka and Kaeriyama 1997). Using Chinook salmon of known age, Bradford and Geen (1992) found that otolith size was correlated with fork length, but the width between otolith increments did not accurately reflect somatic growth. Fisher and Pearcy (1990) validated that scale radius was an accurate predictor of fork length in coho salmon *Oncorhynchus kisutch*, and that growth rate was correlated with width between scale circuli. Fukuwaka and Kaeriyama (1997) reported that scale radius was related to fork length in individually marked, repeatedly sampled sockeye salmon *Oncorhynchus nerka*, and that the width between circuli and somatic growth were correlated. Although no studies have attempted to link somatic growth in salmonids and the width between otolith daily increments and scale circuli, Baumann et al. (2013) compared lengths of haddock *Melanogrammus aeglefinus* that were back-calculated from otolith and scale radii at particular annuli. The authors found that back-calculated lengths differed between otoliths and scales because the first annulus was deposited closer to the origin in scales relative to otoliths.

Validation of the relationship between whole body growth and the growth of hard structures in each species (and possibly stock [see Zabel et al. (2010)] and life stage [see Secor and Dean (1992)]) must occur before any attempts at retrospective growth can be performed accurately. In conjunction with my research, a retrospective growth study using scales from adult Chinook salmon in the Kuskokwim and Yukon river systems was conducted to examine the importance of juvenile freshwater growth (Leon 2013). The author found that freshwater growth had little impact on female productivity, egg

deposition, and age of returning females. Evidence was found for a minimum freshwater length that juvenile fish needed to achieve to survive to adulthood (also known as the threshold size), but the relationship between the threshold size and survival was weak and varied between river systems. While freshwater growth was unrelated to recruitment, the author noted an important caveat that the fish considered in his study were those that survived to adulthood. If scales from fish that had not successfully recruited to the adult population could have been obtained for analysis, stronger relationships between freshwater growth and recruitment might have been uncovered (e.g., Moss et al. 2005). My study serves to validate the assumption from Leon (2013) that a relationship does exist between whole body growth and growth of hard structures in Chinook salmon, and to define the nature of this relationship. Leon (2013) reported the width between the scale focus and the first freshwater annuli in his study, but did not convert these measurements into fish lengths as there was no validation.

My study also provides a means for using retrospective analysis for estimating length of juvenile Chinook salmon, which has many potential benefits for fisheries managers. Through back-calculation, body size of an individual fish at past ages can be estimated, which can help increase understanding of the meaning of retrospective growth. Knowledge of the growth history of a fish will assist researchers in continuing to unravel the complexities surrounding the importance of size attained in freshwater to survival of juvenile salmon both in freshwater and saltwater environments, as well as the effect of freshwater size on recruitment to the adult population (e.g., Neilson and Geen 1986; Holtby et al. 1990; Cross et al. 2008; Ruggerone et al. 2009; Leon 2013). Retrospective growth analysis can also be used to determine if threshold sizes exist for juvenile Chinook salmon and how those threshold sizes may vary from year to year (e.g., Smith and Griffith 1994; Beamish and Mahnken 2001; Beamish et al. 2004).

The goal of my study was to examine the relationship between freshwater growth of juvenile Chinook salmon and the development of growth increments on otoliths and scales. Through a laboratory study, where multiple food ration and stocking density treatments were applied to replicate a range of growing conditions that wild juvenile

salmon might encounter, my objective was to validate the relationship between body size and growth and width between daily otolith growth increments and scale circuli in age-0 Chinook salmon. My specific hypotheses were as follows: (1) otolith and scale circuli increments will be directly dependent on fish growth rate, with growth increments for each structure growing in a constant, measurable proportion to each other; (2) the width between hard structure increments in otoliths and scales will reflect the growth rate of juvenile Chinook salmon; and (3) larger otolith and scale growth increments will be positively correlated with lower fish density and higher food ration. My results will assist fisheries managers by testing the supposition that fish body size, growth, and growth rate are reflected by otolith and scale size and growth-increment formation.

Methods

Fish Rearing

Six hundred eyed Chinook salmon eggs from the Salcha River were obtained from the Alaska Department of Fish and Game (ADF&G) Fort Richardson Hatchery on October 1, 2010. Upon arrival, eggs were disinfected in a 10% solution of povidone iodine (Western Chemical, Ferndale, Washington) for 10 minutes and were placed in vertical-stack hatching trays on top of a 520-L raceway. The raceway, which served as a biofilter, was filled with biosphere media and populated with ammonia- and nitrite-removing bacteria. Water percolated through the hatching trays, collected in the raceway, and flowed into two, 197-L sump tanks where it was chilled using two submersible D1-100 chillers (Frigid Units, Toledo, Ohio). Water was returned to the heath trays using a 1.5-hp inline pump.

The eggs and hatched larvae were maintained at 10^{o}C in flows of approximately 23 L/min until they had hatched (eggs) and used approximately two thirds of their yolk sac (larvae). At this point, larvae were moved to a 326-L fry insert installed in a 900-L circular tank until they had reached the swim-up stage, at which point a high protein finfish starter meal was fed *ad libitum* to the fish on a daily basis (55% crude protein, 15% crude fat, 1% crude fiber; Ziegler Brothers, Gardners, Pennsylvania). Larger

crumble sizes were progressively offered as fish increased in size, but all diets were nutritionally identical. Water quality was checked daily to maintain an acceptable environment for fish, which included measurements of temperature (10^{o} - 12^{o} C), salinity (2 - 4 ppt), dissolved oxygen (8 – 12 mg/L), free ammonia-N (0.02 - 0.06 mg/L), and free nitrite-N (0.004 - 0.008 mg/L; Westin 1974; Weatherley and Gill 1995; Kelsey et al. 2002; Quinn 2005). Temperature, salinity, and dissolved oxygen were measured using an YSI 85 Dissolved Oxygen/Conductivity Instrument (YSI Incorporated, Yellow Springs, Ohio), while free ammonia-N and free nitrite-N were tested using an Aquaculture Test Kit (Hach Company, Loveland, Colorado).

Fish were held in the hatchery tanks until the majority of individuals reached 60 to 70 mm in total length. Prior to the beginning of the laboratory experiment, fish were randomly selected and placed into 24, 110-L aquaria as part of a recirculating aquaculture system (Aquatic Habitats, Apopka, Florida). The system consisted of four stainless steel frames that each held six aquaria. Flow rates were maintained at 3.75 L/min, and each aquarium was drained by two standpipes into four, 800-L settling tanks. The settling tanks were seeded with biospheres and ceramic media, and were populated with the ammonia- and nitrite-removing bacteria as described previously for the hatching/rearing system biofilter. These tanks drained into a 285-L raceway, where the water was chilled by four submersible chillers, and returned to the aquaria using a 1.5-hp inline pump.

Experimental Design

On March 17, 2011, 360 randomly selected fish were anesthetized in a 70-ppm solution of tricaine methanesulfonate (MS-222). Each fish was measured for total length (TL) to the nearest 1 mm and weighed to the nearest 0.1 g (wet weight; WW). Following measurement, each fish was marked with one or more visual implant elastomer tags (VIE; Northwest Marine Technologies, Shaw Island, Washington). Marks were applied by injecting the elastomer material with a 0.3-ml insulin syringe equipped with a 29-gauge needle. The needle was inserted into the fish just under the skin or at the base of the fin, and elastomer was injected to create the mark. Marks were applied in a manner

to allow identification of individual fish in each aquarium, although marks were repeated among aquaria. The combination of two colors in conjunction with five body locations served to allow for the individual identification of 20 different fish. One fish in each tank received no mark to act as a control. Marked fish received VIE tags in the adipose tissue behind either eye or at the base of the dorsal, anal, and/or caudal fin (Table 1).

To examine the role of density on fish growth and otolith- and scale-increment formation, 12 aquaria each received 10 fish and 12 aquaria were each stocked with 20 fish. The same water-quality conditions described for the hatchery system were maintained in the experimental system. Experimental fish were fed a high protein pellet (50% crude protein, 15% crude fat, 2% crude fiber; Zeigler Brothers, Gardners, Pennsylvania), with three feeding regimes assigned to simulate a low growth ration (1% of fish body weight), a maintenance ration (2% of fish body weight), and a high growth ration (4% of fish body weight). Density of fish and feeding regimes were assigned at random for a 2 X 3 block design (density x feeding ration), with four replicates per treatment.

Fish were allowed to acclimate to experimental conditions for 14 d, at which point a photoperiod was fixed at 18h light: 6h dark and the temperature regime typified streams at 65^{o}N latitude during spring and summer months. Uneaten food and fecal material were siphoned out of each tank on a daily basis. Dead fish were removed when discovered (n = 4 during the experimental period), measured for total length and wet weight as described previously, and frozen for later laboratory analysis. Every 30 d, all fish were anesthetized, measured for total length, and weighed for wet weight as described previously. At the conclusion of the experiment (d 122), all fish were sacrificed, measured for total length and wet weight, and frozen for subsequent laboratory analyses.

Laboratory Analyses

Sagittal otoliths were removed from each fish and stored in individual centrifuge tubes, allowed to dry for 7 d, and mounted on microscope slides in Crystal Bond 509

Table 1. Color, body location, and assignment of visual implant elastomer marks to tanks based on stocking density in Chinook salmon selected for the present growth-increment study.

Mark color(s) and body location(s)	Treatment
No mark	10 + 20 Fish
Green left eye	10 + 20 Fish
Green right eye	10 + 20 Fish
Green anal fin	10 + 20 Fish
Green caudal fin	10 + 20 Fish
Green dorsal fin	10 + 20 Fish
Red left eye	10 + 20 Fish
Red right eye	10 + 20 Fish
Red caudal fin	10 + 20 Fish
Red dorsal fin	10 + 20 Fish
Red anal fin	20 Fish Only
Green anal fin + red caudal fin	20 Fish Only
Green anal fin + red dorsal fin	20 Fish Only
Green anal fin + green caudal fin	20 Fish Only
Green anal fin + green dorsal fin	20 Fish Only
Green anal fin + green caudal fin + green dorsal fin	20 Fish Only
Red anal fin + red caudal fin	20 Fish Only
Red anal fin + red dorsal fin	20 Fish Only
Red anal fin + green caudal fin	20 Fish Only
Red anal fin + green dorsal fin	20 Fish Only

(Structure Probe, Inc., West Chester, Pennsylvania). An image of each otolith was taken using a digital camera mounted on a Leica compound microscope (Leica Microsystems, Wetzlar, Germany), and the maximum diameter and radius were measured for each otolith using Image-Pro Plus (Version 7.0; Media Cybernetics, Inc., Rockville, Maryland). Otoliths were ground and polished with lapping film until the daily growth rings were clearly discernable. Prepared otoliths were photographed using the digital camera system described above using light microscopy at 400X magnification, stitched together using the Leica Application Suite program (Leica Microsystems, Switzerland), and imported into Image-Pro Plus for analysis. Using a macro program designed by ADF&G's Mark, Tag, and Age Laboratory (Juneau, Alaska), an axis for increment counting and measurement was drawn from the most posterior primordia to the edge in the posterior-dorsal quadrant of the otolith (Figure 1). Starting from the edge and counting inward, the first 122 increments (representing those increments deposited during the 122-d experimental period) were marked and the widths between those increments measured. This process was repeated by a second reader. Discrepancies between the counts or measurements of the readers for any otolith were re-examined and resolved, producing a single data set for analyses.

Ten to 15 scales were removed from behind the dorsal fin, immediately above the lateral line of each fish (DeVries and Frie 1996), and dry mounted between two microscope coverslips held together with super glue. The first non-regenerated scale encountered was selected for analysis and photographed using the digital camera system previously mentioned. Each scale's increments were counted by a single reader without knowledge of the experimental treatment. The reader also measured the distance between each scale circuli. A second reader repeated the process, after which both counts and measurements were compared. Discrepancies between the counts or measurements of the readers for any scale were re-examined and resolved, producing a single data set for analyses.

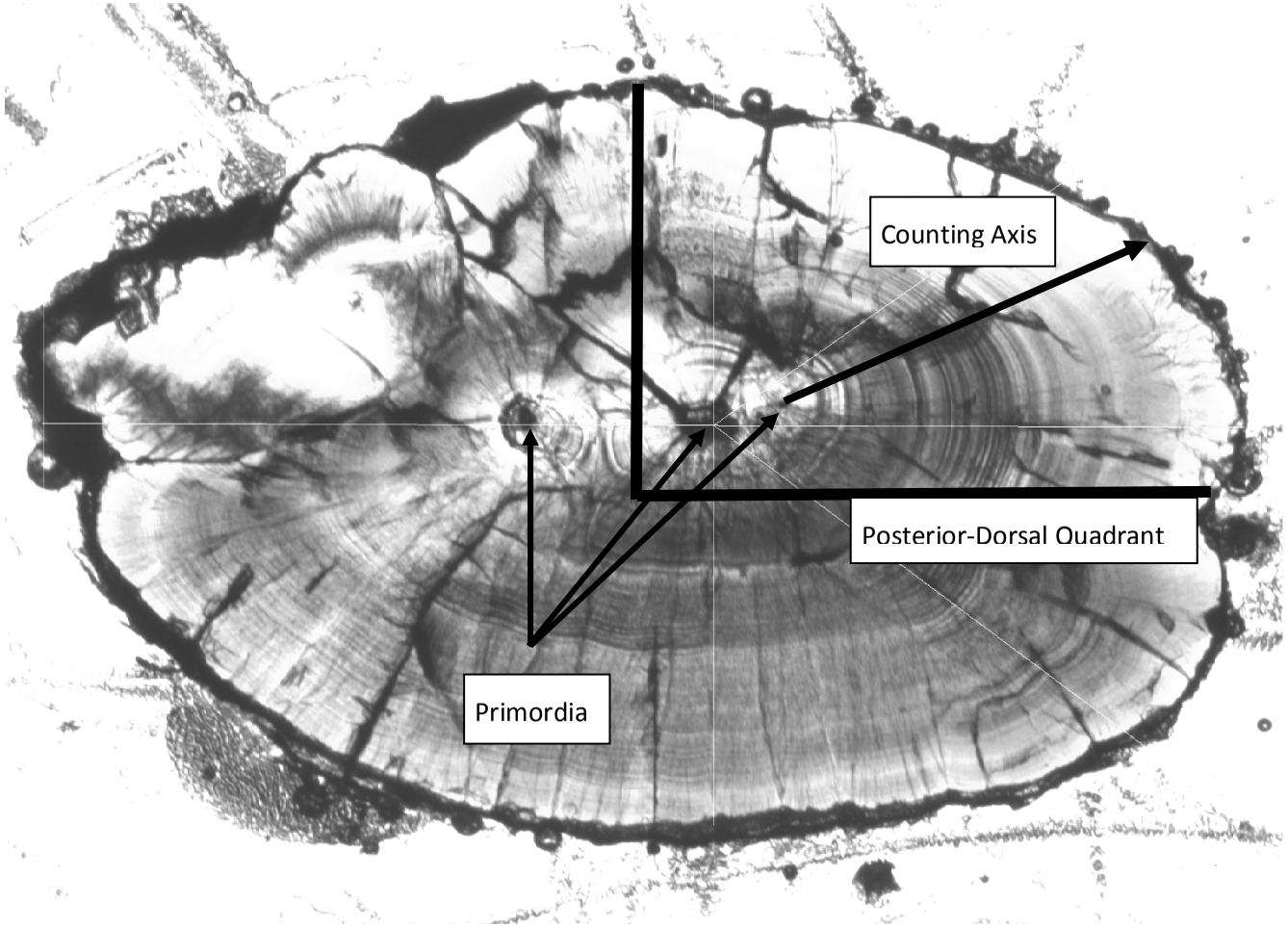

Figure 1. Juvenile Chinook salmon otolith (100X magnification), showing the posterior-dorsal quadrant. Three primordia are identified, with a counting axis drawn from the most posterior primordia to the edge of the otolith.

Data Analyses

The repeated measures and block design of the laboratory experiment resulted in a grouped data set. A linear mixed-effects model was selected to examine the effects of ration and density on fish growth in length and wet weight during the experiment; this type of model incorporates an additional error term (random effects) to account for correlation between observations on the experimental units (each tank) being subjected to the treatments (Pinheiro and Bates 2000). A two-way Analysis of Variance (ANOVA) was conducted to compare the mean length and wet weight of each treatment group at the beginning of the experiment, with a second two-way ANOVA used to compare the mean lengths and wet weights of the treatment groups at the experiment's conclusion. Growth in TL and WW was compared between marked and unmarked (control) fish with a Welch two-sample t-test. Mean TL and WW of each treatment group (dependent variables) were plotted against experimental day (independent variable) to examine the growth performance of each group over time.

Instantaneous growth rate (IGR), estimated as the percentage of change in TL per day, was described as:

$$IGR = [\ln(L_2) - \ln(L_1)]/t,$$

where L_1 was the TL at time 1, L_2 was the TL at time 2, and t was the time elapsed (in days) between length measurements. The mean IGR for each tank was estimated between every measuring event and, using the mean TL at each measuring event along with the corresponding IGR, mean daily lengths were computed for each tank for the duration of the experiment.

Because scale-circuli formation and width between circuli are highly correlated with fish growth and size (Healey 1982; Wootton 1998; Beamish et al. 2004; Moyle and Cech 2004), the relationships between scale radius (independent variable) and TL (dependent variable), scale radius (independent variable) and the number of scale circuli (dependent variable), and TL (independent variable) and the number of scale circuli (dependent variable) were assessed through linear regression. Comparing the model prediction for length at the formation of each scale circuli against the mean daily fish

length for each tank, I was able to extrapolate the mean date of formation for scale circuli developed during the experimental period. Using these dates, the number of otolith daily growth increments deposited and growth in length of the otolith axis was calculated for the time period between the formation of each scale circulus.

The mean number of otolith growth increments formed between the deposition of each scale circulus (dependent variable) and mean growth in otolith axis between the deposition of each scale circulus (dependent variable) were separately regressed against fish total length at the deposition of each circulus (independent variable). Pearson's product-moment correlation analyses were conducted to determine if the number of otolith daily growth increments formed and growth in otolith axis during the time period between deposition of each scale circulus was correlated with mean water temperature. To determine how growth rate was reflected by otolith daily growth increments, the mean width between each otolith daily growth increment formed between the deposition of each scale circulus (dependent variable) was regressed against fish total length at the deposition of each circulus (independent variable). The relationship between the mean width among scale circuli (dependent variable) and TL was also examined using linear regression.

The mean cumulative width between otolith daily growth increments (independent variable) and mean growth in TL (dependent variable) for each tank was compared on a monthly basis using linear regression. For each tank, linear regression was used to examine the relationship between mean scale radius growth (independent variable) and growth in TL (dependent variable). Mean proportional growth, defined as the ratio of scale radius to otolith axis, was calculated at the date of formation of each circulus and was regressed against fish total length at the date of circulus formation (independent variable). For this relationship, a reciprocal transformation was required for the dependent variable. The relationship between otolith axis (independent variable; defined as the length of the counting axis from the posterior most primordia to the edge of the otolith) and TL (dependent variable), otolith diameter (independent variable) and TL (dependent variable), and scale radius (independent variable) and TL (dependent

variable) was assessed using linear regression, where the slope and intercept parameters for each structure were used to create back-calculation models for TL at previous daily ages (Carlander 1981). A measurement of otolith radius (the greatest distance from the posterior-most primordia to the otolith's edge) was examined, but was found to be highly correlated with otolith diameter ($r^2 = 0.701$, $p < 0.001$) and was excluded from subsequent analysis due to redundancy. Total length for each fish at the conclusion of the experiment, as predicted by the otolith diameter and scale radius models (dependent variables), was regressed against the measured TL for each fish (independent variable) at the end of the experiment to compare the accuracy of the back-calculation models against each other.

Residual plots, Q-Q plots, and/or tests for constant variance were examined or conducted in all cases where linear regression was used to ensure that heteroscedacity and normality assumptions were satisfied. Because most back-calculation models assume that the researcher is provided with a hard structure with which to predict the length of the respective fish (e.g., Harvey et al. 2000; Ross et al. 2005), all back-calculation plots were oriented otolith or scale size as the independent variable. Statistical analyses were conducted using *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna, Austria) and *SigmaStat Version 3.5* (Systat Software, Inc., Point Richmond, California) at $\alpha = 0.05$.

Results

Growth

Mean TL of juvenile Chinook salmon was 75.89 mm (SE = 0.308; range = 55 – 94 mm) prior to the beginning of the experiment and 110.76 mm (SE = 0.502, range = 81 – 146 mm) at the experiment's conclusion. There was no significant difference in TL among treatment groups at the beginning ($F = 0.975$, $p = 0.378$) or conclusion ($F = 0.436$, $p = 0.647$) of the experiment. Growth during the experimental period in TL was not significantly different between marked and unmarked (control) fish ($t = 1.573$, $p = 0.127$). The treatment group fed a 2% body weight ration and stocked at a tank density of

10 fish grew to the largest mean TL (115.49 mm), while fish fed at 4% of body weight at a density of 20 fish per tank had the smallest mean TL (108.73 mm; Figure 2a; Table 2). Fish in all treatment groups grew in TL and WW over the experiment. Neither density ($F = 1.46$, $p = 0.241$) nor ration ($F = 1.45$, $p = 0.259$) had a significant effect on TL; however, TL was significantly related with experimental day ($F = 3060.97$, $p < 0.001$), the interaction term for density x experimental day ($F = 13.38$, $p < 0.001$), and the interaction term for ration x experimental day ($F = 18.85$, $p < 0.001$). Total length at each measuring period was highly correlated to TL at the previous measuring period (correlation coefficient = 0.936). Model predictions of mean TL for each treatment group at each measuring period were within ± 2 mm of mean TL for that period (Table 3).

Mean WW for experimental fish was 3.97 g (SE = 0.051; range = 1.5 – 7.8 g) at the beginning of the experiment and 12.49 g (SE = 0.184; range = 3.2 – 27.3 g) at the experiment's conclusion. There was no significant difference in WW at either the beginning ($F = 0.784$, $p = 0.485$) or conclusion ($F = 0.627$, $p = 0.535$) of the experiment. Growth during the experimental period in WW was not significantly different between marked and unmarked (control) fish ($t = 1.956$, $p = 0.06$). The treatment group fed a 2% body weight ration and stocked at a tank density of 10 fish grew to the largest mean WW (14.26 g), while the group fed at 4% of body weight at a density of 20 fish per tank had the smallest mean weight (11.74 g; Table 4; Figure 2b). Neither density ($F = 4.162$, $p = 0.0548$) nor ration ($F = 2.467$, $p = 0.1103$) had a significant effect on WW; however, WW did have a significant relationship with experimental day ($F = 1657.968$, $p < 0.001$), the interaction term for density x experimental day ($F = 7.63$, $p < 0.001$), and the interaction term for ration x experimental day ($F = 13.119$, $p < 0.001$). Wet weight for each measuring period was also highly correlated to WW measured at the previous measuring period (correlation coefficient = 0.929). Model predictions of mean WW for each treatment group at each measuring period were within ± 1 g of the measured mean WW (Table 5).

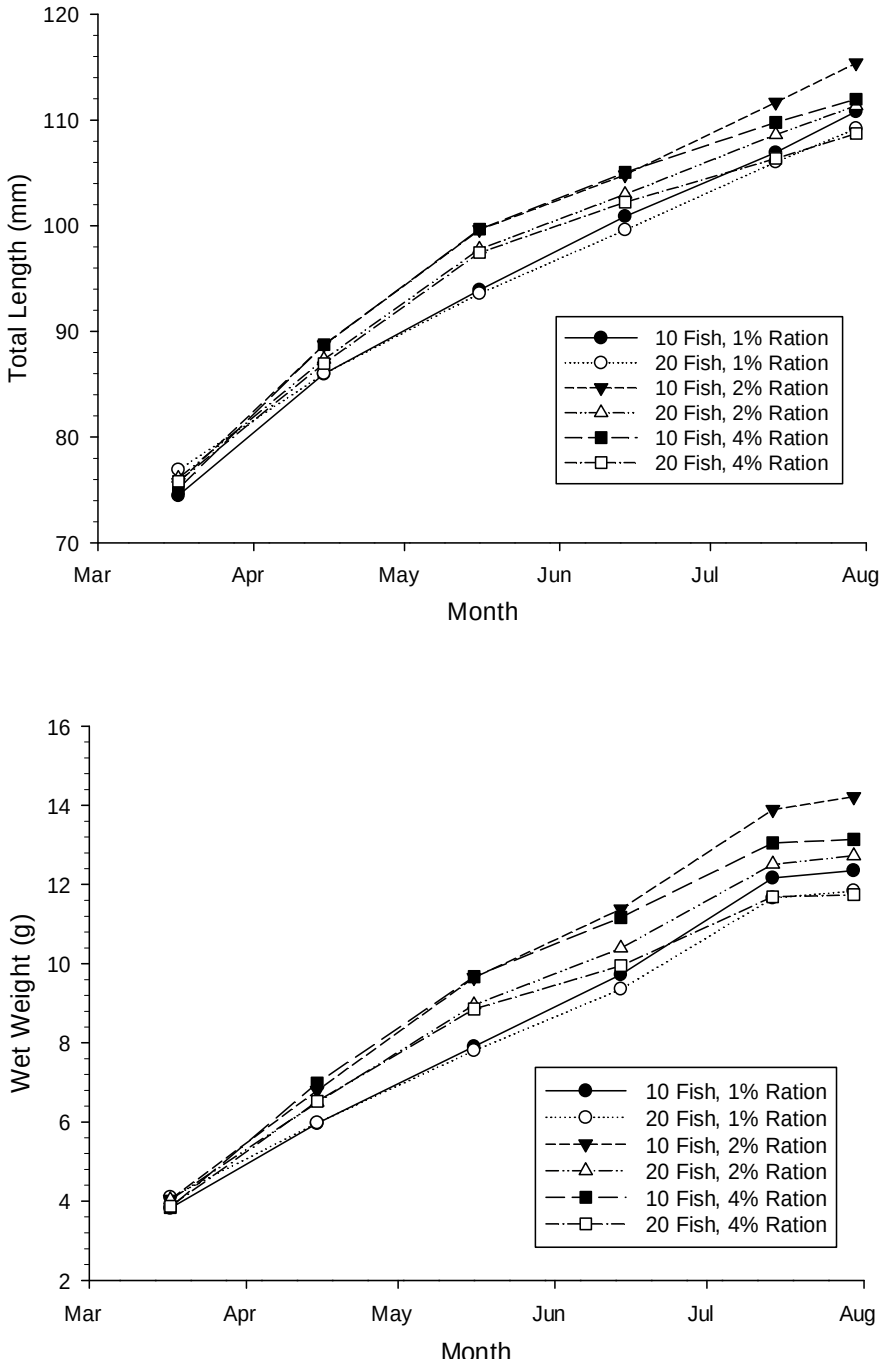

Figure 2. Growth in mean TL (a) and mean WW (b) of each treatment group of juvenile Chinook salmon over the duration of the experiment.

Table 2. Mean total length (mm) of experimental juvenile Chinook salmon for each density x ration treatment with standard deviations for each value in parentheses. Total length at marking was measured during marking and stocking 14 d prior to the start of the experiment.

			Day				
Density	Ration (% body weight per day)	Length at Marking	16	46	76	106	122
10	1%	74 (5)	86 (6)	94 (6)	101 (7)	107 (8)	111 (8)
10	2%	76 (6)	89 (6)	100 (7)	105 (7)	112 (8)	115 (8)
10	4%	75 (6)	89 (6)	100 (8)	105 (8)	110 (9)	112 (10)
20	1%	77 (5)	86 (6)	94 (7)	100 (7)	106 (8)	109 (9)
20	2%	76 (6)	87 (8)	98 (9)	103 (10)	109 (11)	111 (12)
20	4%	76 (7)	87 (7)	97 (8)	102 (9)	106 (9)	109 (10)

Table 3. Mean total length (mm) of experimental juvenile Chinook salmon for each density x ration treatment as predicted by the linear-mixed effects model.

			Day				
Density	Ration (% body weight per day)	Length at Marking	16	46	76	106	122
10	1%	75	87	95	101	108	112
10	2%	75	89	100	105	111	115
10	4%	75	89	100	105	110	112
20	1%	76	85	92	98	104	107
20	2%	76	87	97	102	108	111
20	4%	76	87	97	102	106	108

Table 4. Mean wet weight (g) of experimental juvenile Chinook salmon for each density x ration treatment with standard deviations for each value in parentheses. Wet weight at marking was measured during marking and stocking 14 d prior to the start of the experiment.

			Day				
Density	Ration (% body weight per day)	Weight at Marking	16	46	76	106	122
10	1%	3.8 (0.9)	6.0 (1.4)	7.9 (1.6)	9.8 (2.1)	12.2 (2.9)	12.4 (3.0)
10	2%	4.0 (0.9)	6.8 (1.3)	9.7 (1.8)	11.4 (2.1)	13.9 (2.7)	14.3 (2.8)
10	4%	3.8 (1.0)	7.0 (1.7)	9.7 (2.5)	11.2 (3.0)	13.1 (3.6)	13.1 (3.9)
20	1%	4.1 (0.9)	6.0 (1.5)	7.8 (1.9)	9.4 (2.4)	11.7 (3.1)	11.8 (3.3)
20	2%	4.0 (0.9)	6.5 (2.0)	9.0 (2.9)	10.4 (3.5)	12.5 (4.2)	12.7 (4.4)
20	4%	3.9 (1.1)	6.6 (1.8)	8.9 (2.4)	10.0 (2.6)	11.7 (3.3)	11.7 (3.4)

Table 5. Mean wet weight (g) of experimental juvenile Chinook salmon for each density x ration treatment as predicted by the linear-mixed effects model.

			Day				
Density	Ration (% body weight per day)	Weight at Marking	16	46	76	106	122
10	1%	4.0	6.2	8.3	10.1	12.6	12.8
10	2%	4.0	6.8	9.6	11.4	13.7	14.0
10	4%	3.8	7.0	9.7	11.1	13.0	13.1
20	1%	4.0	5.8	7.6	9.1	11.4	11.6
20	2%	4.1	6.4	8.9	10.4	12.5	12.7
20	4%	3.9	6.7	9.0	10.1	11.9	11.9

Scale Analyses

A significant positive linear relationship was found between Chinook salmon TL and the number of scale circuli ($r^2 = 0.431$, $p = < 0.001$; Figure 3a). Scale circuli counts ranged from 11 to 27, with a mean of 20.32 (SE = 0.138). Model results predicted that circuli 15 through 21 were formed during the 122-d experimental period, with fish depositing a new circulus at each successive increase of 5 to 6 mm in TL. The number of scale circuli deposited on a scale was strongly related to scale radius ($r^2 = 0.55$, $p < 0.001$; Figure 3b), and scale radius was significantly correlated to fish TL ($r^2 = 0.538$, $p < 0.001$; Figure 3c).

Otolith Analyses

Although the number of otolith increments deposited between the formation of each circulus and Chinook salmon TL yielded a moderate r^2 value of 0.442, this relationship was not significant ($p = 0.09$; Figure 4a). Otolith growth between circulus deposition was also not significantly related to fish TL ($r^2 = 0.296$, $p = 0.153$; Figure 4b). These relationships were not linear, following a similar trend to the experimental water temperature profile (Figure 4c). The Pearson's correlation coefficient for the mean number of otolith increments formed between deposition of each circulus and average water temperature was 0.811, while the coefficient between otolith growth and temperature was slightly stronger (0.819). Both the mean width between daily otolith increments ($r^2 = 0.948$, $p = < 0.001$; Figure 5a) and the mean width between scale circuli ($r^2 = 0.964$, $p = < 0.001$; Figure 5b) had a strong negative linear relationship with TL.

Growth in otolith axis length between measuring periods was significantly related to mean growth in fish TL ($r^2 = 0.36$, $p < 0.001$; Figure 6a). Mean growth in TL and mean scale radius growth were also significantly related to each other ($r^2 = 0.449$, $p < 0.001$; Figure 6b), indicating that growth in otolith axis and scale radius was proportional to growth in fish TL. However, mean proportional growth between otoliths and scales exhibited a significant negative linear relationship with fish TL ($r^2 = 0.918$, $p <0.001$; Figure 6c), demonstrating that otoliths and scales do not grow in constant proportion to each other.

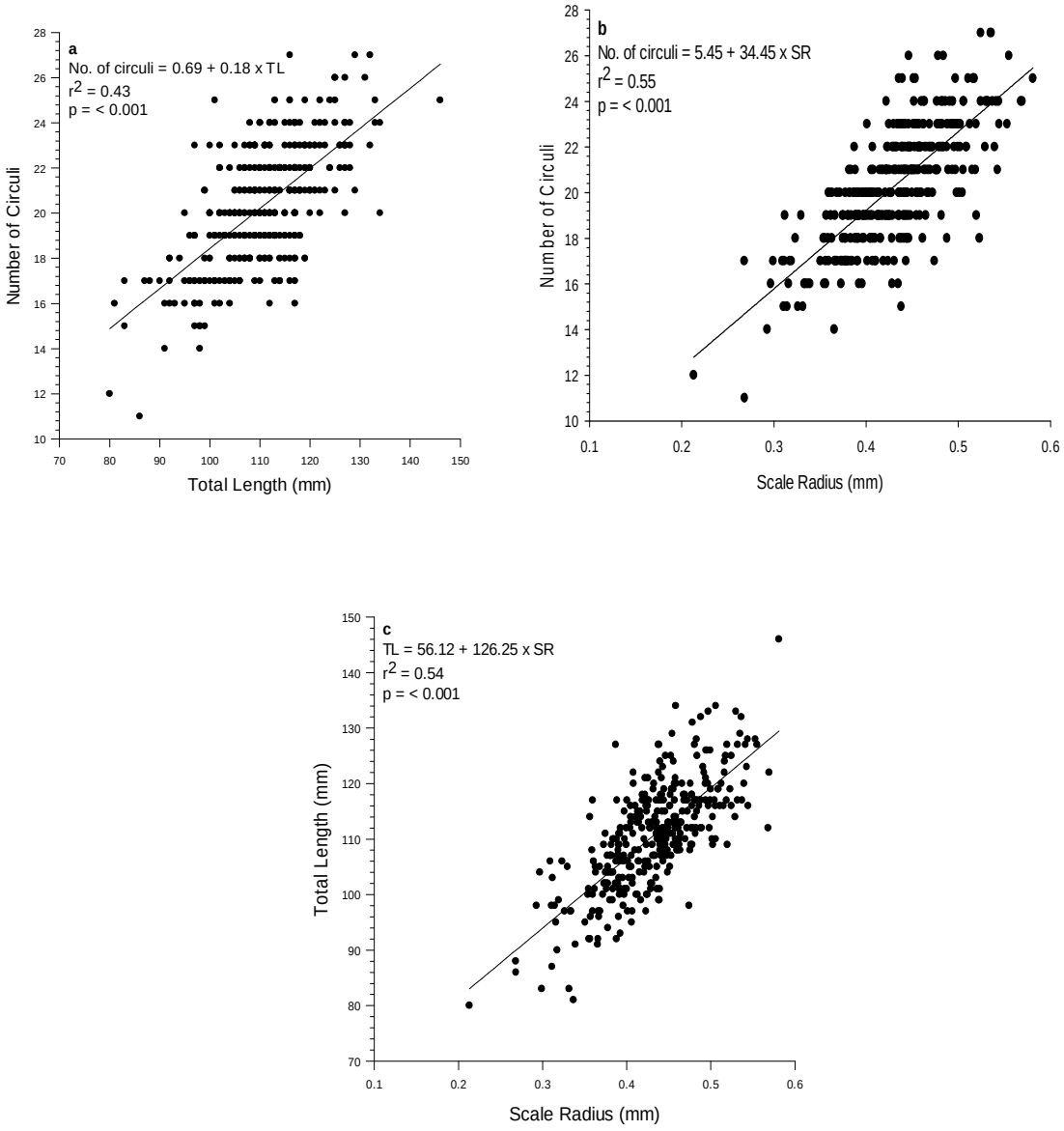

Figure 3. Relationships between (a) the number of circuli deposited on a scale and fish total length (TL; mm), (b) the number of circuli deposited on a scale and scale radius (SR; mm), and (c) fish total length (TL; mm) and scale radius (SR; mm) for juvenile Chinook salmon. For all plots, the regression line is the predicted value of the dependent variable at a given value of the independent variable.

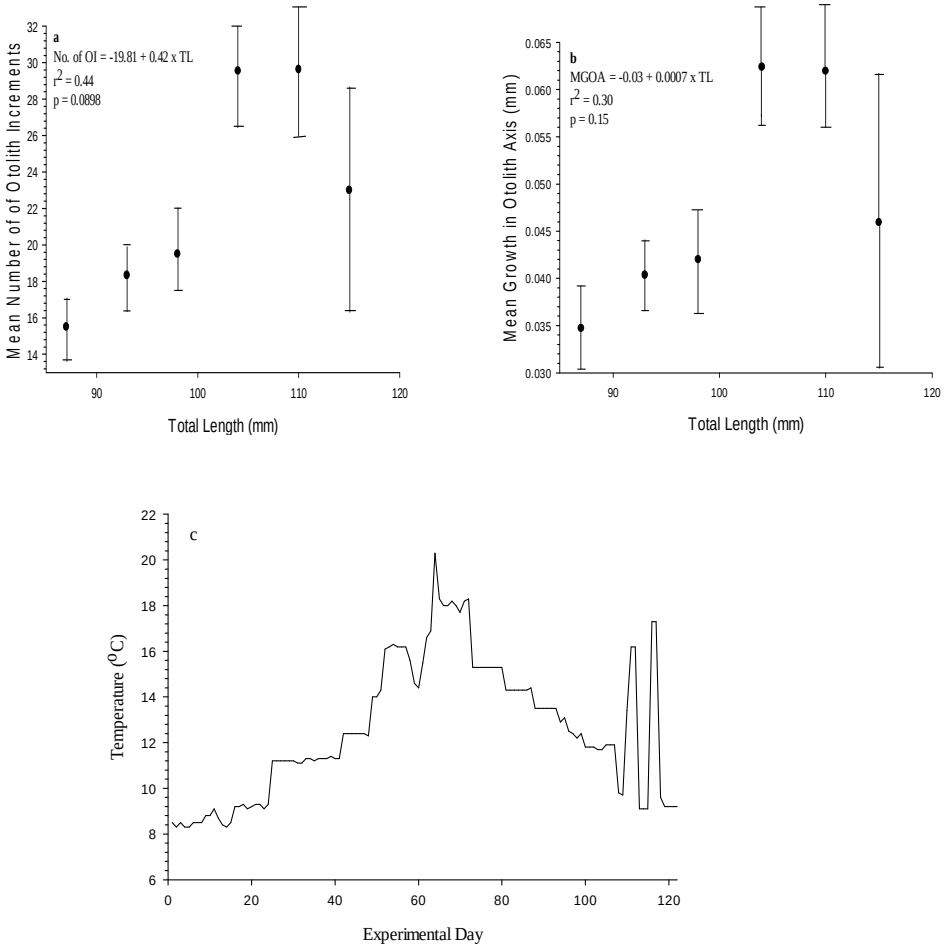

Figure 4. Relationships between (a) the mean number of otolith increments (OI) formed between deposition of scale circuli and fish total length (TL, mm) at the time of circuli deposition, and (b) mean growth in otolith axis (MGOA; mm) between the deposition of scale circuli and fish total length (TL; mm) at the time of circuli deposition. Length values represent the mean length at which each scale circuli modeled to be formed under experimental conditions was deposited. For both plots, error bars represent 95% confidence intervals. Plot (c) is the temperature profile experienced by Chinook salmon during the experiment. Temperature spikes near the conclusion of the experiment represent attempts to induce otolith thermal marks.

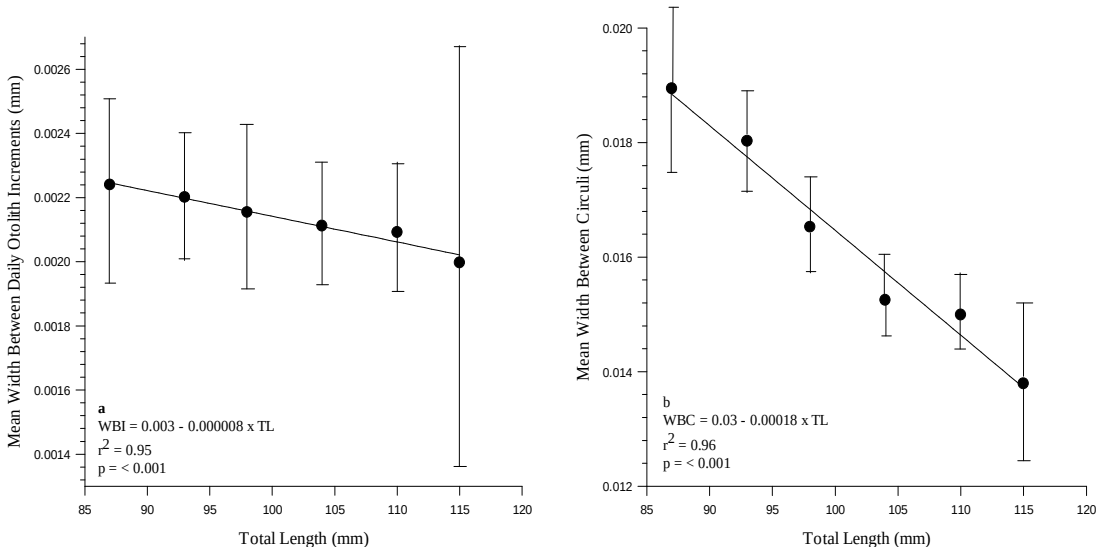

Figure 5. Relationships between (a) mean width between each daily otolith increment (WBI; mm) formed between scale circuli deposition and fish total length (TL; mm) at the time of circuli deposition, and (b) mean width between each scale circuli (WBC; mm) modeled to be formed at experimental conditions and fish total length (TL; mm). Length values represent the mean length at which each scale circuli modeled to be formed under experimental conditions was deposited. For all plots, error bars represent 95% confidence intervals, and regression lines are the predicted value of the dependent variable at a given value of the independent variable

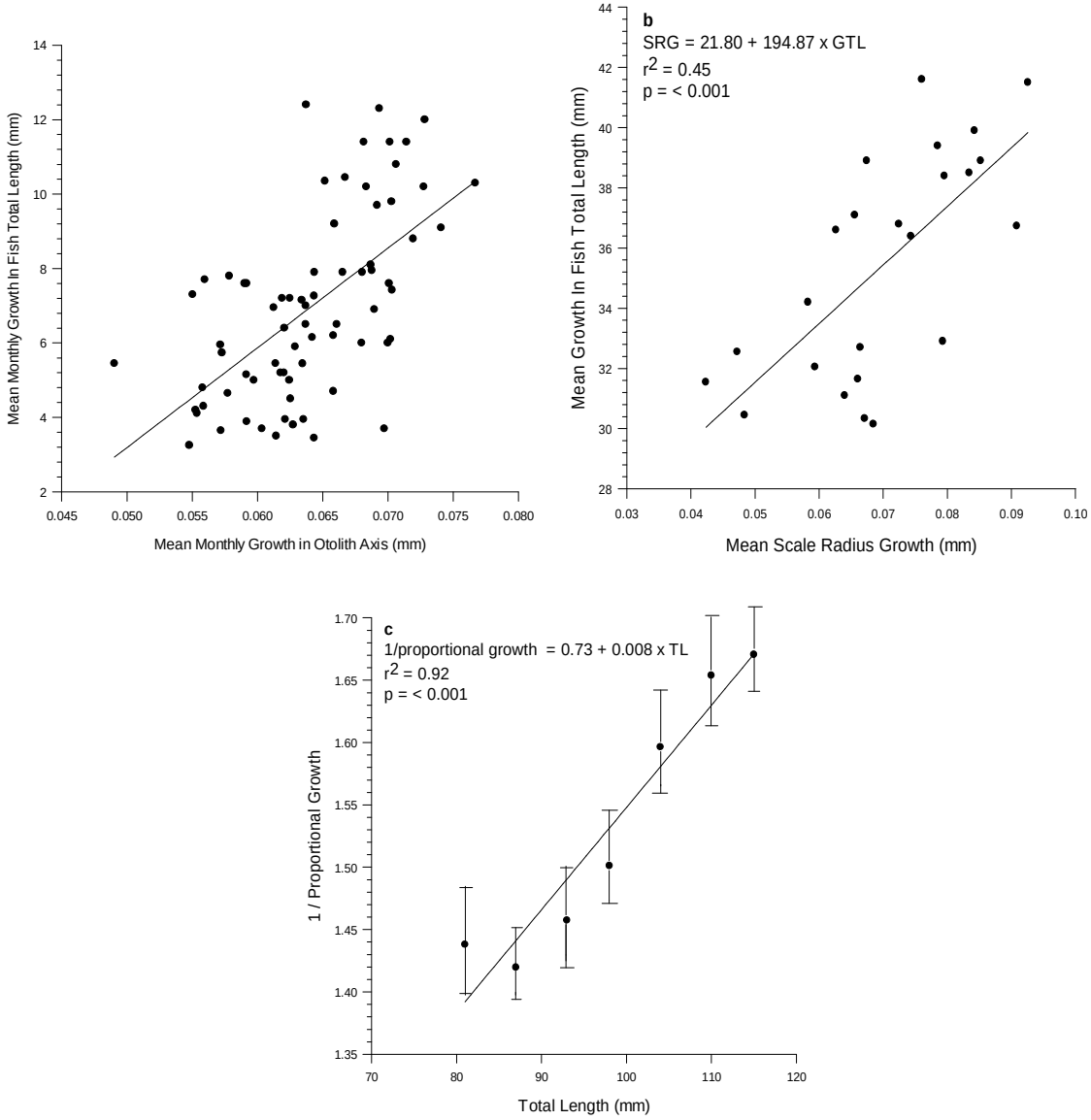

Figure 6. Relationships between (a) mean monthly growth in fish total length (mm; MGFTL) and mean monthly growth in otolith axis (MGOA; mm), (b) mean growth in fish total length (GTL; mm) and mean scale radius growth (SRG; mm), and (c) 1/proportional growth and fish total length (TL; mm) of experimental juvenile Chinook salmon. For all plots, error bars represent 95% confidence intervals, and regression lines are the predicted value of the dependent variable at a given value of the independent variable.

Back-calculation Models

Length of the otolith axis was significantly correlated with fish TL ($r^2 = 0.209$, $p < 0.001$; Figure 7a). The back-calculation model constructed for otolith axis fit the data poorly, with deviations from observed lengths as much as 30.11 mm (29.98%). Otolith diameter was more strongly associated with TL ($r^2 = 0.667$, $p < 0.001$; Figure 7b). Although the back-calculation model for otolith diameter better fit the relationship with fish TL, it still deviated from observed total lengths by as much as 16.70 mm (15.73%). As noted previously, scale radius had a significant positive linear relationship with TL, with the scale radius back-calculation model deviating from observed TL by as much as 21.94 mm (21.93%).

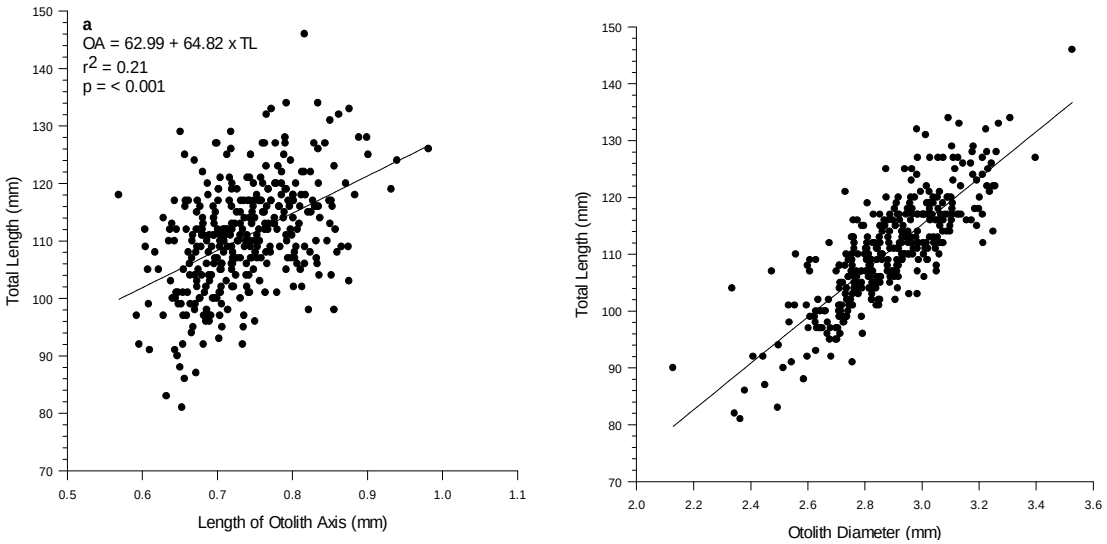

Figure 7. Relationships between (a) fish total length (TL; mm) and the length of otolith axis (OA; mm) and (b) fish total length (TL; mm) and otolith diameter (OD; mm) for juvenile Chinook salmon. The regression line is the predicted total length based on the length of otolith axis or otolith diameter.

Discussion

Ration and density treatments imposed on juvenile Chinook salmon in the present study did not produce significant differences in TL and WW among treatment groups. However, there was a significant relationship between TL/WW and time (experimental day), along with significant interactions with density x time and ration x time. The significant relationship between TL/WW and time is likely due to the positive growth in TL and WW exhibited by each fish during the experiment. Interactions between density x time and ration x time indicate that density and ration influenced growth, but their effect was not consistent. Abiotic factors, particularly water temperature, have a complex influence on the impact of density and ration on fish growth (Wootton 1998; Crozier et al. 2010). For example, Crozier et al. (2010) found that higher fish densities negatively impacted growth, and this effect intensified with an increase in water temperature. Water temperature can also influence the effect of ration on fish growth; when fish cannot feed at their maximum rate, increases in water temperature can result in reduced growth rates (Wootton 1998). Lower rations can also result in a narrower range of water temperatures at which a fish can experience positive growth, and reduce the optimum temperature for growth under those conditions (McNab 2002; Crozier et al. 2010). As a result, interactions between density x time and ration x time likely reflect the changing temperature regime experienced by the fish during the experiment.

Mean fish growth rates for each tank ranged from 0.25 to 0.34 mm/d in TL and 0.053 to 0.094 g/d in WW over the duration of the experiment. Although these results are low for hatchery-reared fish, they are within the reported ranges for other hatchery-reared and natural-origin stocks of stream-type Chinook salmon. For example, Beckman et al. (1998) observed growth rates of 0.12 g/d in WW for age-0 hatchery-reared spring Chinook salmon from September through November, with growth rates increasing from February through mid-June to 0.25 g/d. Beckman et al. (1999) reported growth rates of 0.067 mm/d for age-1 Chinook salmon parr from February through late April at Warm Springs National Fish Hatchery, Oregon, but also noted fish from Round Butte Hatchery, Oregon, that grew at a rate of 0.53 mm/d over the same period. Brett et al. (1982)

observed mean growth rates of wild spring Chinook salmon fry to be 0.066 g/d in the Nechako River, British Columbia, while Healey (1991) reported growth rates for wild Chinook salmon yearling smolts that ranged from 0.077 to 0.33 mm/d in TL for several populations ranging from Idaho to Alaska. Hill and Webber (1999) found growth rates of 0.45 to 1.13 mm/d in a mark-recapture experiment for wild age-0 spring Chinook salmon in Butte Creek, California. Similar results have also been reported for juvenile coho salmon (Fisher and Pearcy 1990; Ruggerone and Rogers 1992; Jones et al. 2011). Because growth rates of age-0 Chinook salmon in the present study were similar among treatments, factors other than food ration most likely influenced the results.

Growth of Chinook salmon during the laboratory experiment may have been affected by innate behaviors, as the fish were hatched from gametes harvested from wild parents. Fish crowded to the rear of the aquaria and/or scattered when the aquaria were approached by people. Further, fish were reluctant to feed while food was being delivered to nearby aquaria, and only fed normally once I moved out of their direct line of sight. In other studies, fish from domesticated strains have been observed to be more aggressive in feeding and less wary than wild conspecifics (Swain and Riddell 1990; Johnsson and Abrahams 1991; Berejikian 1995). For example, Sutton et al. (2002) and Volkman et al. (2004) found that brook trout *Salvelinus fontinalis* from two newly domesticated strains (less than a decade in the hatchery) grew more slowly than fish from a domestic strain that had been developed 40 years prior. The authors in these studies noted that anthropogenic movement associated with delivering food caused fish from the newly domesticated strains to elicit a startle response and crowd to the back of the tanks, while the domesticated fish reacted to the disturbance of food delivery by aggressively feeding and competing with each other for food. Negus (1999) observed similar results for natural-origin steelhead *Oncorhynchus mykiss* and a strain of domesticated rainbow trout *Oncorhynchus mykiss*. Hatcheries indirectly select for fish that are more aggressive and less wary of predators because fish that are competitively superior for food tend to grow the largest in size (Johnsson and Abrahams 1991). Fish that aggressively feed without concern of predation exhibit the best growth rates in a protected hatchery

environment, whereas these behaviors in a natural setting would likely result in mortality (Berejikian 1995). Fish that are comparatively larger than their conspecifics often survive to adulthood at a greater rate, resulting in subsequent generations being increasingly better adapted for survival in a hatchery environment (e.g., more aggressive, less wary; Reisenbichler and Rubin 1999). As a result, it is probable that the lower than expected growth rates observed in the present study were due to an undomesticated (i.e., wild) strain of fish.

Stocking density did not have a significant relationship with Chinook salmon TL in the present experiment, but competition among fish for food may have exercised a biologically significant influence on fish growth. Total length among fish in each aquarium ranged from 13 to 54 mm at the conclusion of the experiment, with some aquaria exhibiting noticeable size differences among fish (e.g., a few large fish and many medium or small individuals). Fish TL was also strongly correlated with otolith size, which has been shown to be correlated with metabolic rate and dominance (Titus 1990; Titus and Mosegaard 1991; Metcalfe et al. 1992; Metcalfe et al. 1995; Yamamoto et al. 1998). Food pellets were distributed as equally as possible at the tank surface, but the largest fish in each aquaria were observed to feed directly under the opening where food was delivered. Smaller fish were nipped, chased, and driven into the corners and away from the primary location that feed was delivered, suggesting that a dominance hierarchy had been established (Newman 1959; Keenleyside and Yamamoto 1962; Stein 1971; Abbott et al. 1985; McMichael et al. 1999; Kelsey et al. 2002). Juvenile stream-type salmonids are territorial, with dominant fish securing and defending the most profitable feeding locations (McMichael 1999; Quinn 2005). Fenderson et al. (1968) observed that a single dominant Atlantic salmon *Salmo salar* parr consumed as much or more than three subordinate parr in the same aquarium. Metcalfe et al. (1992) found that dominant Atlantic salmon grew more quickly than subordinates, and that dominant fish were more efficient in converting food into somatic growth. Social dominance in salmonids has been linked to comparatively higher metabolic rates (Metcalfe et al. 1992; Metcalfe et al. 1995; Yamamoto et al. 1998), which allows fish a greater scope for growth and activity

due to being able to more quickly process consumed prey items and extract energy (Titus 1990). A more rapid rate of digestion and elimination allows fish to consume more food items than other individuals over the same time period (Titus and Mosegaard 1991), permitting fish with a higher metabolism to grow more quickly and reach larger lengths than conspecifics (Titus 1990; Titus and Mosegaard 1991; Metcalfe et al. 1992). Larger body size has been shown to enhance the dominance hierarchy, in that the larger the disparity in size, the greater the ability for large fish to secure, defend, and control feeding opportunities and territories (Wootton 1998). As a result, the social interactions in the present experimental aquaria may have prevented subordinate fish from being able to feed effectively and further contributed to the low mean growth rates and small body size observed in this study.

Use of a slow-sinking feed type may have also hindered growth of juvenile Chinook salmon. Pellets were consumed by the fish at the water surface and in the water column, but were largely ignored once they had settled to the bottom. Due to the shallow depth of the aquaria, pellets sank to the bottom within 2 to 3 minutes. Previous studies have shown that presenting food too rapidly (i.e., sinking through the feeding zone too quickly) can result in significant wastage of feed (Juell et al. 1994; Ang and Petrell 1998). Talbot et al. (1999) found that caged Atlantic salmon (200 – 4,000 g) required 5 to 25 minutes to feed to satiation at a variety of feed delivery rates, and observed that time to satiation increased with increasing ration size. Brett (1971) observed that sockeye salmon required approximately 45 minutes to feed to satiation, with comparatively smaller fish requiring more time. Nagata (1989) found that age-0 masu salmon *Oncorhynchus masou* reached satiation at about 60 minutes, and noted that time to satiation increased with fish body size. Fish in the latter two studies were similar in size to experimental Chinook salmon in the present study, suggesting that the fish did not have sufficient time to consume their ration before it became unavailable to them.

The feed pellet size used in the present experiment may also have adversely influenced Chinook salmon growth rates. Food pellets were appropriate in size for juvenile Chinook salmon at the beginning of the experiment, but fish should have been

switched to a larger pellet as they increased in TL. As fish grow, optimum prey (or particle) size increases (Werner 1974; Wankowski and Thorpe 1979; Linner and Brannas 1994). Fish consuming comparatively larger prey can feed more efficiently because fewer prey items need to be captured to reach satiation, large prey provide more energy (calories) than comparatively smaller food items, handling time is reduced, and the fish is exposed to predators for shorter periods during foraging (Werner 1974; Werner and Hall 1974; Keeley and Grant 2001; Daly et al. 2009; Duffy et al. 2010). Fish that consume a diet of small prey items require additional feeding time due to having to identify, pursue, and capture each food item (Werner 1974; Werner and Hall 1974; Talbot et al. 1999). Using a commercially prepared dry feed, Juell et al. (1994) found that by increasing food pellet size by 50%, feeding time was reduced from 50 to 70% for Atlantic salmon. Use of progressively larger pellets may have allowed Chinook salmon in the present study to ingest greater quantities of feed per unit time, making it possible for fish to consume more of their daily ration before it settled onto the bottom of the aquaria and became unavailable to them. Increasing food consumption has been shown to increase growth and growth rates (Bilton and Robins 1971a; Wootton 1998), and should have allowed juvenile Chinook salmon to achieve greater size during experimental conditions.

The present study results suggest that somatic growth may not be the primary factor determining otolith increment size. Overall otolith size was significantly correlated with fish body size, but daily growth was not accurately reflected by the width between the corresponding daily otolith increments. As an example, mean growth in fish total length between the April and May measuring periods was 9.71 mm, slowed to 5.56 mm from May to June (a decrease of 43%), and was steady at 5.55 mm from June to July. Over the same time, growth in otolith axis length was 0.069 mm between April and May, slightly decreasing to 0.064 mm between May and June (a decrease of 7.2%), and declining to 0.060 mm from June to July. Previous research suggested that otolith increment width is more strongly controlled by metabolic rate than by somatic growth (Mosegaard et al. 1988; Wright et al. 1990; Bradford and Geen 1992; Yamamoto et al. 1998; Wright et al. 2001). Somatic growth rate and metabolic rate of fish are closely

related, but do not always occur at a similar rate (Wootton 1998). Because fish are poikilotherms, increases in water temperature lead to increases in metabolic rate (Weatherley and Gill 1987). These increases in metabolic rate require more energy, which necessitates that the fish expend more of its consumed resources to maintain current size and status (McNab 2002). As long as fish can feed at their maximum rate, increases in water temperature will result in increases in growth rate until the optimum temperature for growth is reached (Handeland et al. 2008). At water temperatures exceeding the optimum for fish growth, growth rates decline as the fish's body shifts energy from somatic growth to support necessary biological functions due to the increases in metabolic activity (Wootton 1998). As a result, a disparity forms between somatic growth and metabolic rates.

During periods of differential growth and metabolic rates, the relationship between otolith size and fish body size often becomes uncoupled (Mosegaard et al. 1988). The authors showed that otolith increment widths increased with increasing water temperature, even as somatic growth rate slowed in Arctic char *Salvelinus alpinus*. Yamamoto et al. (1998) found a significant positive correlation between metabolic rate and daily otolith growth increment width in masu salmon. Wright et al. (1990) observed that the width between daily otolith increments remained constant over time for Atlantic salmon following the cessation of somatic growth. Further, Wright et al. (2001) found that the width between otolith increments and daily oxygen consumption (used as a proxy for standard metabolic rate) increased for fasting fish when water temperature was raised from 5 to 15^{o}C. In the latter two studies, neither the length nor weight of fish increased during the experimental periods, indicating that somatic growth did not occur. However, uncoupling is not permanent; proportionality between otolith size and growth and fish size and growth is gradually restored once metabolic rate is no longer disproportionate to somatic growth rate (Maillet and Checkley 1990; Bradford and Geen 1992; Secor and Dean 1992; Bestgen and Bundy 1998).

Compensatory growth in otoliths should be expected because this structure is a key element of fish hearing and balance (Campana 1999). As a fish increases in size, the

otolith, sensory epithelium, and size of cilia bundles all proportionally increase in size or density to allow hearing and vestibular sensitivity to remain constant (Campana 2004; Popper et al. 2005). Maillet and Checkley (1990) showed that daily otolith increments continued to form and that the width between increments decreased over time in fasting Atlantic menhaden *Brevoortia tyrannus*. However, the width between increments gradually increased once feeding was allowed to resume. Bradford and Geen (1992) found that increment widths in juvenile Chinook salmon gradually decreased under a restrictive ration, producing negligible somatic growth. When full ration was restored, width between otolith increments gradually increased, but remained more narrow than increment widths displayed by fish in the control group (continuously fed full ration) through the termination of the experiment.

A large body of evidence suggests that otolith size (e.g., diameter, radius, and mass) and growth are related to fish body size and growth (e.g., Bradford and Geen 1992; Secor and Dean 1992; Harvey et al. 2000; Strelcheck et al. 2003; Sakaris and Irwin 2008). The present study found that otolith diameter was significantly related to fish total length in juvenile Chinook salmon. Growth in length of the otolith axis was also significantly correlated with growth in fish total length on a monthly basis. Bradford and Geen (1992) found that otolith increments did not reliably reflect somatic growth in juvenile Chinook salmon, but there was a significant linear relationship between otolith radius and fish length. Secor and Dean (1992) showed that otolith diameter was strongly correlated with fish length in striped bass *Morone saxatilis*. Harvey et al. (2000) reported observed relationships between fish length and otolith diameter for 63 species of Pacific Ocean fishes (including Chinook salmon), with otolith diameter explaining at least 70% of the variability in fish length for 45 species (including Chinook salmon [$r^2 = 0.99$]). This relationship between fish length and otolith diameter was significant for all but one species, jack mackerel *Trachurus symmetricus*. Strelcheck et al. (2003) showed a positive relationship between otolith diameter and standard length ($r^2 > 0.75$) in captive-reared and wild populations of juvenile gag *Mycteroperca microlepsi*. Further, otolith mass was a better predictor of fish length for gag, with r^2 values as high as 0.97. Sakaris

and Irwin (2008) found correlations greater than 0.90 for the relationship between otolith radius and total length in a study of larval channel catfish *Ictalurus punctatus*. Based on the present results and similar studies (see also Campana and Neilson [1985]), it appears that the width between otolith increments did not accurately reflect fish growth on a short-term basis (e.g., daily), but otolith size and otolith growth did accurately reflect fish size and growth over longer periods of time (e.g., monthly).

Chinook salmon scale radius in the present study was significantly correlated with fish TL and the number of scale circuli. My research is the first study to validate these relationships for Chinook salmon, although they have been described previously for other salmon species (coho salmon: Fisher and Pearcy 1990; sockeye salmon: Fukuwaka and Kaeriyama 1997). Beamish et al. (2004) found a strong relationship between scale length (radius) and fish length in coho salmon. Cross et al. (2008) and Fukuwaka (1998) confirmed a similar association between scale radius and fork length for pink salmon *Oncorhynchus gorbuscha* and sockeye salmon. Healey (1982) found scale radius to be a strong predictor of fish length for juvenile chum salmon *Oncorhynchus keta*, and observed that the number of scale circuli increased in proportion to the size of the scale and fish size. Similar results were observed for the relationship between scale radius, fish length, and the number of scale circuli for coho salmon (Fisher and Pearcy 2005) and pink salmon (Courtney et al. 2000). Validation of the use of scale radius as a predictor of fish length in Chinook salmon confirms the utility of retrospective growth analyses for this species.

Scale growth reflected somatic growth for Chinook salmon in the present experiment, with a significant linear relationship between mean growth in scale radius and mean growth in fish total length. The width between circuli was wider in comparatively faster growing experimental Chinook salmon, with a similar relationship between circuli width and fish growth rate confirmed for coho salmon (Bilton and Jenkinson 1977). In the present study, the width between individual circuli accurately reflected somatic growth rate as mean width between individual circuli decreased linearly over time. This result was corroborated by my calculations of mean IGR which also

decreased over time. Fisher and Pearcy (2005) found a similar decrease in growth rate for coho salmon parr as the fish increased in age and size. Additionally, the authors (1990) observed a strong correlation between circuli width and growth rate for coho salmon following ocean entry. Fukuwaka (1998) noted a similar correlation between circulus spacing and growth rate for chum salmon and sockeye salmon. The present study is the first known evaluation to confirm that scale growth accurately reflects somatic growth in Chinook salmon.

For Chinook salmon in this experiment, deposition of scale circuli appeared to be governed primarily by increases in fish size (somatic growth), with circuli being formed as needed to allow scale size to increase with fish size. The present result agrees with the findings by Healey (1982; chum salmon), Fisher and Pearcy (1990; coho salmon), Fukuwaka and Kaeriyama (1995; sockeye salmon), Courtney (2000; pink salmon), and Fisher and Pearcy (2005; coho salmon), where the frequency of scale circuli deposition was correlated with fish size and/or growth rate. For the experimental fish, no relationship was found between increment formation in otoliths and circulus formation in scales, either on a temporal basis or as a result of an increase in otolith size. While otolith increments were deposited daily, there was no fixed timing of scale circuli formation. In addition, no relationship existed between otolith growth and the frequency of scale circulus deposition. While otolith growth was proportional to somatic growth and scale growth was proportional to somatic growth, otolith growth was not proportional to scale growth. Kruse et al. (1997) found that otoliths and scales yielded greatly different back-calculated lengths for juvenile (age-1) Yellowstone cutthroat trout *Oncorhynchus clarkii*, but that the difference in back-calculated lengths between structures became much smaller as age increased. Baumann et al. (2013) found that the first annulus is deposited closer to the origin in scales relative to otoliths in haddock, and that the two structures produced different back-calculated lengths. Based on the preceding examples, it is plausible that otoliths and scales grow proportionally following squamation and that the disproportionate relationship between the structures is a result of the later development of scales. This supposition was not supported by Baumann et al.

(2013), as correcting for length at squamation could not harmonize the back-calculated lengths generated from otolith and scale models. The authors suggested that the factors influencing otolith increment formation (metabolic rate) and scale circuli deposition (somatic growth) are different and growth of the two structures proceeds independently from each other.

Somatic growth regulates circulus formation, but may not be the only factor that determines whether or when a circulus is deposited. Bilton and Robins (1971a) found that feed ration was significantly correlated with the number and spacing of scale circuli in sockeye salmon. The authors (1971b; 1971c) also observed that while no circuli were formed, photoperiod may influence circuli spacing and scale growth. Starved fish held under continuous light or darkness exhibited increases in scale radius and inter-circuli widths, even though there was no somatic growth. Fisher and Pearcy (1990) showed that coho salmon age and size may affect the rate of circuli formation, whereas Bilton (1970) found that water temperature influenced the rate of circuli deposition. Whether these and other abiotic factors directly influence circuli formation in fish is a matter of conjecture because they also directly impact somatic growth.

For the back-calculation models generated in the present experiment, otolith diameter was a better predictor of fish length, followed by scale radius and otolith axis. The difference in performance of otolith diameter and scale radius was relatively minor in that scale radius deviated from observed lengths by about 5 mm (6%) more than otolith diameter (Figure 1.8). Given the difference in preparation effort and time required to prepare an otolith for examination versus using a scale sample, the sacrifice in resolution through the use of scales is acceptable. Using scales in retrospective growth analyses is non-lethal, and allows subject fish to be sampled repeatedly over time (see Fisher and Pearcy 1990). Scales can be used to accurately age juvenile Chinook salmon (McNicol and MacLellan 2010), although they may be less precise than other structures for adult Chinook salmon (e.g., fin rays; Copeland et al. 2007).

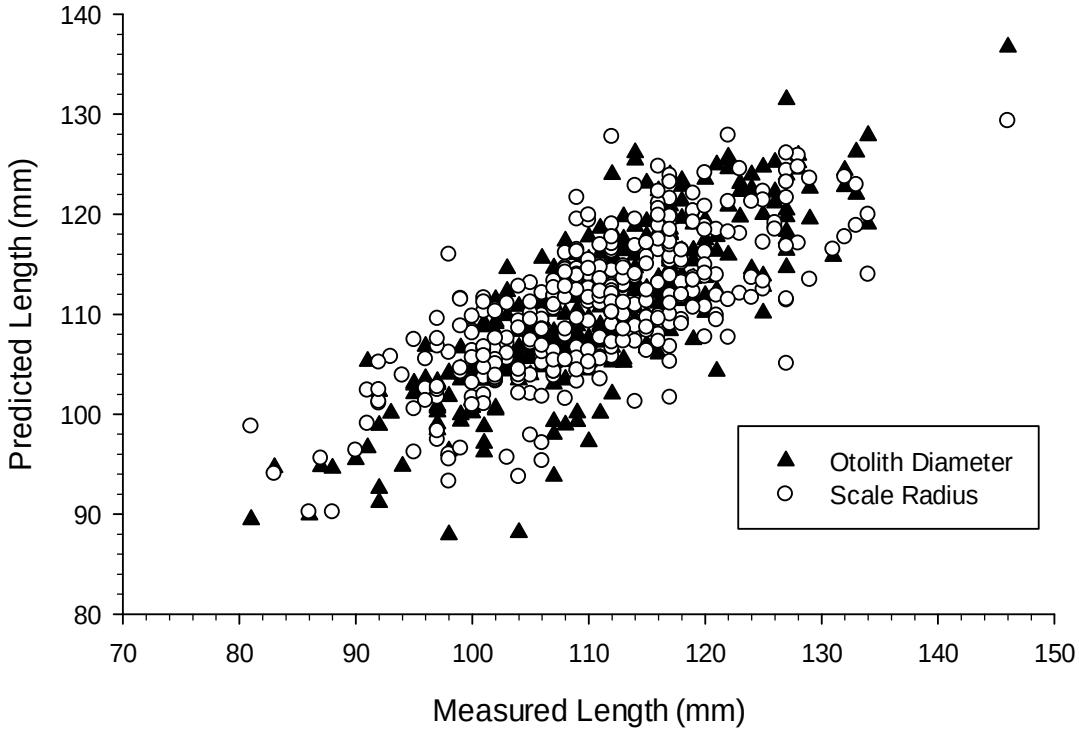

Figure 8. Comparison of predicted lengths from the otolith diameter and scale radius back-calculation models against the actual length of each experimental Chinook salmon.

This result agrees with previous research involving other salmonids, which found that aging of fish based on scales is accurate for juveniles, but is less reliable for older individuals following the onset of sexual maturity (Sikstrom 1983; Sharp and Bernard 1988; Ericksen 1999; Maceina et al. 2007; Muir et al. 2008; Stolarski and Sutton 2013). The present results showed that scales can be used as an acceptable alternative to otoliths in retrospective growth analyses involving juvenile Chinook salmon.

Following analysis of the study results, several caveats were identified in the present methodology and study approach. First, otolith axis length exhibited an unexpectedly weak correlation with Chinook salmon TL. Neilson (1984) noted that salmonids have a randomly scattered pattern of primordia in the otolith nucleus, which makes the nucleus vary in shape and size among individual fish. In attempting to establish a fixed point from which to begin my otolith axis measurements (the most posterior primordia), I may have inadvertently introduced error into the measurement of total otolith axis length. Campana and Neilson (1985) confirmed the variance in the number and position of otolith primordia and in nucleus size among individuals, but Neilson (1984) found that otolith increment widths were not affected by these differences. Meekan et al. (1998) observed a similar weak relationship between otolith radius and TL in Atlantic salmon ($r^2 = 0.17$), but found that using otolith area as a proxy for otolith radius improved the relationship with TL ($r^2 = 0.44$). Replacing otolith axis with a measurement of otolith area may have produced a more consistent relationship with TL in the present study due to eliminating the error associated with measuring from a randomly located primordia. Second, scale samples for each fish should have been taken at the beginning of the experiment, and possibly at each measuring period. This would have allowed me to determine the number of circuli formed by each fish during experimental conditions, as well as to more accurately compare scale growth and somatic growth over time. Fisher and Pearcy (1990) took multiple scale samples over time from their experimental coho salmon and were able to more precisely compare somatic and scale growth rates. Finally, induction of otolith thermal marks at the beginning of the

experiment would have been helpful in confirming those otolith increments formed under experimental conditions. Thermal marking was attempted, but this type of marking requires rapid changes in water temperature (Hagen et al. 1995) and we were unable to lower the water temperature quickly enough to produce recognizable marks in the experimental fish.

My results successfully validated a proportional relationship between otolith size (radius and diameter) and fish TL, as well as a proportional relationship between scale size (radius) and fish TL in juvenile Chinook salmon. In addition, I was able to demonstrate that the width between scale circuli accurately reflected somatic growth, and that growth in size of both the otolith and scale were proportional to somatic growth. These findings provide a solid foundation for future retrospective growth analyses involving Chinook salmon, which will help in assessing the importance of size attained by juvenile salmon in freshwater to subsequent survival and recruitment to the adult population. My results will allow calculation of juvenile Chinook salmon TL from the size of otoliths and scales. As noted previously, Leon (2013) could not convert his measurements of freshwater scale growth into measurements of fish lengths as there was no validation. The results of my study do allow the conversion of otolith and scale measurements into fish lengths, which are meaningful in studies of survival and recruitment. Back-calculated lengths can be used in retrospective growth analyses to detect the existence of threshold sizes for populations of juvenile Chinook salmon, and to determine how those threshold sizes vary from year to year. Smith and Griffith (1994) demonstrated the existence of a threshold size for rainbow trout in freshwater environments, while Beamish and Mahnken (2001) and Beamish et al. (2004) found that coho salmon needed to attain a minimum size in order to survive their first winter at sea. Presumably Chinook salmon would also be subject to similar size-selective environmental constraints, which retrospective analysis of surviving fish would help to uncover (e.g., Leon 2013).

Retrospective analysis can help to elucidate the strength of size-selective predation on cohorts of juvenile Chinook salmon, especially during the critical period

immediately following ocean entry (e.g., Healey 1982; Sogard 1997; Beamish and Mahnken 2001; Cross et al. 2008). Growth rates can also be estimated and subsequently compared with smolt migration (e.g., Beckman et al. 1998), early marine growth (e.g., Beamish and Mahnken 2001) and ocean residency (e.g., Ruggerone et al. 2009) to determine how the rate of growth affects the timing and rapidity of emigration from freshwater, early marine survival, and the length of time a fish spends at sea. The mean growth rate of a cohort can also be compared to the corresponding cohort in previous years to determine what effect growth rate has on recruitment. My results validated the use of scales in retrospective growth analyses of juvenile Chinook salmon, allowing managers an accurate, non-lethal alternative to otoliths in reconstructing fish size at age and past growth histories.

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Appendix

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June 14, 2010

To: Trent Sutton, PhD
Principal Investigator

From: University of Alaska Fairbanks IACUC

Re: [170553-3] Effect of Ichthyophonus infection on Yukon River Chinook salmon hatching success, growth, and survival

The IACUC reviewed and approved the New Project referenced below by Designated Member Review.

Received:	June 8, 2010
Approval Date:	June 14, 2010
Initial Approval Date:	June 14, 2010
Expiration Date:	June 14, 2011

This action is included on the July 1, 2010 IACUC Agenda.

The PI is responsible for acquiring and maintaining all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol, and could result in revocation of IACUC approval.